# Chemical imaging of functional group distributions in living biofilms using infrared microspectroscopy

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## INTRODUCTION

Bacteria predominantly grow as biofilms attached to liquid or solid interfaces. Biofilms are complex structures that contain bacterial cells interwoven in an extracellular polysaccharide matrix. The protein, lipid and polysaccharide molecules within a biofilm contain characteristic functional groups which can be identified and mapped using infrared microspectroscopy. The objective of this study is to image the distributions of biomolecules within living biofilms and to monitor the chemical evolution of biofilms *in situ* during microbial growth.

## METHODS

Infrared maps were collected for *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* biofilms on the Infrared Beamline 1.4.3. at the Advanced Light Source (Lawrence Berkeley National Lab). Biofilms were grown on IR reflective slides in tryticase soy broth for 48 h. Infrared images were collected with an unmasked beam focused through the infrared microscope with a spot size of 10x10 um. The beamline has an incident IR energy range of 0.05 to 1.0 eV, which is nondestructive to bacterial cells. All IR spectra were recorded in the mid-IR range from 4000-650 $cm^{-1}$, which contains unique molecular fingerprint-exhibiting vibrational frequencies of biomolecular functional groups.

## RESULTS

Figure 1 illustrates the spatial distribution of protein, lipid and polysaccharride biomolecules within a *K. pneumoniae* biofilm. Characteristic spectral features of these molecules were observed between 900 and 3000 $cm^{-1}$. In the double bond region (2000-1500 $cm^{-1}$) the spectra show two primary features that arise from specific stretching and bending vibrations associated with protein molecules. For both bacteria species, we observe the amide I feature at 1650 $cm^{-1}$ and the amide II band at 1540 $cm^{-1}$. The spectra also display a characteristic lipid peak at 2960 $cm^{-1}$ due to asymmetric -$CH_3$ vibrational frequencies. Polysaccharide peaks were also detected over a 300 wavenumber range, between 1200 and 900 $cm^{-1}$.

## DISCUSSION

Our results indicate that infrared microspectroscopy can be applied to detect micro-colony formation at the 10-100 μm scale. The presence and distribution of protein molecules can be readily imaged due to the strong amide I and II spectral peaks. *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* displayed weak polysaccharide spectral features, and therefore the distribution of polysaccharide molecules were difficult to image. Because infrared light is nondestructive to biological materials, future studies will be aimed at applying this technique to probe the changes in functional chemistry during biofilm growth.

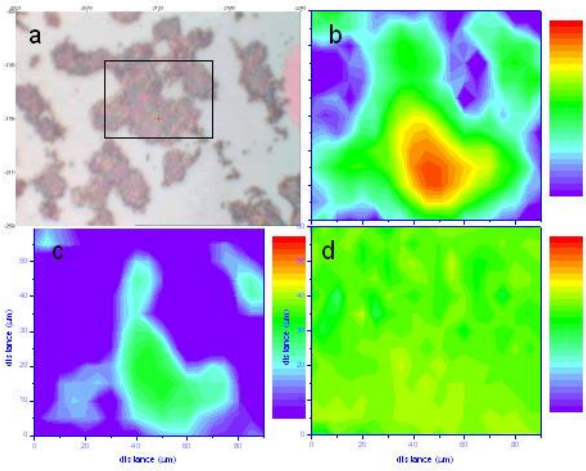


Figure 1. Chemical image of biomolecules within a *Klebsiella pneumoniae* biofilm; a) optical image of the biofilm (the rectangle represents the region mapped); b) proteins (amide I at 1650 $cm^{-1}$); c) lipids 2960 $cm^{-1}$; and d) polysaccharides (1150-1000 $cm^{-1}$).

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